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Analysis of HLA-DRB1 Alleles in Japanese Patients With Chronic Myelogenous Leukemia

Masaki Yasukawa,[1]* Hideki Ohminami,[1] Kensuke Kojima,[2] Koiti Inokuchi,[3] Yasuharu Nishimura,[4] and Shigeru Fujita[1]

[1]First Department of Internal Medicine, Ehime University School of Medicine, Ehime, Japan
[2]Division of Hematology, Ehime Prefectural Central Hospital, Ehime, Japan
[3]Division of Hematology, Third Department of Internal Medicine, Nippon Medical School, Tokyo, Japan
[4]Division of Immunogenetics, Department of Neuroscience and Immunology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan

To clarify the association between HLA-DRB1 alleles and chronic myelogenous leukemia (CML), the HLA-DRB1 allele frequencies in 50 Japanese patients each with b2a2 and b3a2 CML and 127 healthy Japanese individuals were examined. In the patients with b2a2 CML, the frequencies of HLA-DRB1*0405, DRB1*08032, and DRB1*1502 were low and that of HLA-DRB1*1201 was high in comparison with the healthy individuals. The frequencies of HLA-DRB1*0403, DRB1*0802, DRB1*1403, and DRB1*1405 were high, and those of HLA-DRB1*08032 and DRB1*1501 were low in the patients with b3a2 CML. The present results suggest positive and negative associations between certain HLA-DRB1 alleles and CML. Am. J. Hematol. 63:99–101, 2000. © 2000 Wiley-Liss, Inc.

Key words: HLA-DRB1; chronic myelogenous leukemia; bcr-abl fusion protein; CD4$^+$ T lymphocytes; genetic association

INTRODUCTION

It has been demonstrated that the T-lymphocyte-mediated immune response plays an important role in immunosurveillance against chronic myelogenous leukemia (CML). Translocation t(9;22) (q34;q11) has been reported to occur frequently in healthy adults at a very low level [1]. Immunosurveillance for abnormal cells with chromosomal translocation may be one of the reasons for the low frequency of CML development among individuals with t(9;22) (q34;q11). Recently, synthetic peptides spanning the fusion point between bcr and abl have been demonstrated to bind to certain types of HLA class I molecule and induce bcr-abl fusion peptide-specific CD8$^+$ cytotoxic T lymphocytes in vitro [2–5]. Cortes et al. have reported an association between certain HLA class I types and responses to interferon α in patients with CML [6], suggesting the importance of the CD8$^+$ T lymphocyte-mediated immune response in resistance to CML. Induction of CD4$^+$ T lymphocytes that proliferate specifically in response to stimulation with bcr-abl fusion peptide in an HLA class II-restricted manner has been also achieved by stimulating peripheral blood lymphocytes of healthy individuals with synthetic bcr-abl fusion peptide [7–10]. If bcr-abl fusion protein-specific and HLA class II-restricted CD4$^+$ T lymphocytes do play an important role in resistance to the development of CML, then the frequencies of certain HLA class II types in patients with CML should be lower than those in the general population. Conversely, if such CD4$^+$ T lymphocytes support the growth of leukemia cells through the production of growth factors for CML cells in response to leukemia cells, then the frequencies of certain HLA class II types in CML patients should be higher than those in the general population. On the basis of these hypotheses, we analyzed the association between HLA-DRB1 genotypes and two types of CML, b2a2 and b3a2.

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*Correspondence to: Masaki Yasukawa, M.D., Ph.D., First Department of Internal Medicine, Ehime University School of Medicine, Shigenobu, Ehime 791-0295, Japan.
E-mail: yasukawa@m.ehime-u.ac.jp

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TABLE I. HLA-DRB1 Allele Frequencies in Japanese CML Patients and Control Subjects

HLA-DRB1 allele	CML b2a2 ($N = 50$) (%)	CML b3a2 ($N = 50$) (%)	Control ($N = 127$) (%)	P value: b2a2 vs control	P value: b3a2 vs control
DRB1 *0101	6 (12.00)	4 (8.00)	13 (10.23)		
*0102	1 (2.00)	0 (0)	0 (0)		
*0301	1 (2.00)	0 (0)	1 (0.79)		
*0401	2 (4.00)	1 (2.00)	0 (0)		
*0403	2 (4.00)	8 (16.00)	2 (1.57)		0.001
*0404	1 (2.00)	0 (0)	0 (0)		
*0405	6 (12.00)[a]	11 (22.00)	40 (31.50)	0.005	
*0406	2 (4.00)	6 (12.00)	8 (6.30)		
*0407	1 (2.00)	2 (4.00)	0 (0)		
*0410	2 (4.00)	4 (8.00)	2 (1.57)		
*0701	2 (4.00)	1 (2.00)	1 (0.79)		
*0802	5 (10.00)	8 (16.00)	5 (3.94)		0.010
*08032	4 (8.00)	1 (2.00)	26 (20.47)	0.033	0.001
*0901	16 (32.00)	14 (28.00)	36 (28.35)		
*1001	2 (4.00)	1 (2.00)	1 (0.79)		
*1101	4 (8.00)	1 (2.00)	3 (2.36)		
*1201	8 (16.00)	2 (4.00)	8 (6.30)	0.046	
*1202	1 (2.00)	2 (4.00)	5 (3.94)		
*1301	1 (2.00)	2 (4.00)	1 (0.79)		
*1302	5 (10.00)	6 (12.00)	11 (8.66)		
*1401	4 (8.00)	3 (6.00)	6 (4.72)		
*1403	0 (0)	4 (8.00)	1 (0.79)		0.023
*1405	0 (0)	5 (10.00)	0 (0)		0.002
*1406	2 (4.00)	1 (2.00)	0 (0)		
*1407	1 (2.00)	0 (0)	0 (0)		
*1501	10 (20.00)	5 (10.00)	28 (22.05)		0.046
*1502	5 (10.00)	6 (12.00)	28 (22.05)	0.046	
*1601	0 (0)	0 (0)	1 (0.79)		
*1602	0 (0)	0 (0)	3 (2.36)		

[a]The values that differ significantly from those of the control subjects are underlined.

MATERIALS AND METHODS

Reverse transcription polymerase chain reaction (PCR) amplification of specific sequences of the *bcr-abl* fusion gene in CML cells was performed using the primers 5′-GCTTCTCCCTGACATCCGTG-3′ and 5′-GGCCCATGGTACCAGGAGTG-3′. The expected lengths of the amplified bcr-abl cDNAs were 409 bp (b2a2) and 484 bp (b3a2). Genomic DNA was extracted from peripheral blood and bone marrow mononuclear cells. The second exon of the *HLA-DRB1* gene was amplified by PCR using specific primers, and each allele was typed using the restriction fragment length polymorphism method. The frequencies of HLA-DRB1 alleles in 50 Japanese patients each with b2a2 and b3a2 type CML were compared with those of 127 healthy Japanese individuals. The significance of differences was determined by Fisher's exact probability test, and differences at $P < 0.05$ were considered significant.

RESULTS

The HLA-DRB1 allele frequencies in 50 patients each with b2a2 and b3a2 CML and 127 healthy Japanese individuals are shown in Table I. In the patients with b2a2 CML, the frequencies of HLA-DRB1*0405, DRB1*08032, and DRB1*1502 were low ($P = 0.005$, $P = 0.033$, and $P = 0.046$, respectively) and that of HLA-DRB1*1201 was high ($P = 0.046$) in comparison with the healthy individuals. The frequencies of HLA-DRB1*0403, DRB1*0802, DRB1*1403, and DRB1*1405 were high ($P = 0.001$, $P = 0.010$, $P = 0.023$, and $P = 0.002$, respectively), and those of HLA-DRB1*08032 and DRB1*1501 were low ($P = 0.001$ and $P = 0.046$) in the patients with b3a2 CML.

DISCUSSION

In the present study, we demonstrated positive and negative associations between certain HLA-DRB1 alleles and Japanese patients with CML. It has been reported that the bcr-abl fusion peptide can elicit a proliferative response of $CD4^+$ T lymphocytes restricted by HLA-DR antigens [7–10]. In addition, we have reported recently that CML cell colonies increased when CML cells were cultured with b3a2 peptide-specific $CD4^+$ T-lymphocyte clones in a b3a2-specific and HLA-DR-

restricted manner [10]. These data strongly suggest that bcr-abl-specific $CD4^+$ T lymphocytes can recognize the bcr-abl fusion peptide which has been naturally processed and expressed in CML cells in the context of HLA-DR molecules. Accordingly, the association between HLA-DRB1 and CML found in the present study might have resulted from the immune response of bcr-abl-specific $CD4^+$ T lymphocytes against abnormal cells carrying a hybrid *bcr-abl* gene, which have been reported to exist in healthy individuals [1].

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